AWARD NUMBER: W81XWH-16-1-0780

TITLE: Improving Ischemia Reperfusion Injury in Vascularized Composite Tissue Allotransplantation Via Histone Deacetylase Modulation

PRINCIPAL INVESTIGATOR: Matthew H. Levine

RECIPIENT: Trustees of the University of Pennsylvania
Philadelphia, PA 19104

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2017	Annual	30 Sep 2016 - 29 Sep 2017

4. TITLE AND SUBTITLE
Improving Ischemia Reperfusion Injury in Vascularized Composite Tissue Allotransplantation Via Histone Deacetylase Modulation

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0780

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Trustees of the University of Pennsylvania
Philadelphia, PA 19104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This work proposes to investigate the impact of histone deacetylase (HDAC) drug inhibition or deletion on the tolerance of limb warm and cold ischemia reperfusion injury (IRI) in scenarios relevant to limb transplantation using mouse models for experimentation. Limitations in tolerated ischemia times limits the scope of donors that can be considered for any particular vascularized composite allotransplant (VCA) recipient. Mitigating IRI therapeutically would have significant impact on the applicability of VCA to military personnel suffering catastrophic limb or tissue loss.

15. SUBJECT TERMS-
None listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	17	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

This work proposes to investigate the impact of histone deacetylase (HDAC) drug inhibition or deletion on the tolerance of limb warm and cold ischemia reperfusion injury (IRI) in scenarios relevant to limb transplantation using mouse models for experimentation. Limitations in tolerated ischemia times limits the scope of donors that can be considered for any particular vascularized composite allotransplant (VCA) recipient. Mitigating IRI therapeutically would have significant impact on the applicability of VCA to military personnel suffering catastrophic limb or tissue loss.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Vascularized composite allotransplantation (VCA); Histone deactylase (HDAC); ischemia reperfusion injury (IRI); cold ischemia; warm ischemia; mouse; limb transplantation

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

CY16 Goals
Obtain regulatory approval – accomplished (100% complete by Dec 2016)

CY17 Goals
Aim 1 - Develop Warm Limb Ischemia Model (100% complete by May 2017)
- Develop Pathology and Laboratory Based Assays for Limb Injury (100% complete November 2017)
- Test IRI in HDAC knockout and HDAC drug inhibitor treated mice (task 1&2) (50% complete November 2017)

Aim 2 - Develop Cold Limb Ischemia (Transplant) Model (task 1) (100% complete June 2017)

CY18 Goals
Aim 1 – remaining 50% to be complete first half CY 2018
Aim 2 - Complete Cold Ischemia Experiments in HDAC-inhibitor treated mice (task 1) (to begin early CY 2018)
- Complete Cold Ischemia Experiments in HDAC-knockout mice (task 2) (to begin early CY 2018)
- Complete assessment of donor/recipient contribution of cold ischemia tolerance (task 3) (to begin early CY 2018)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

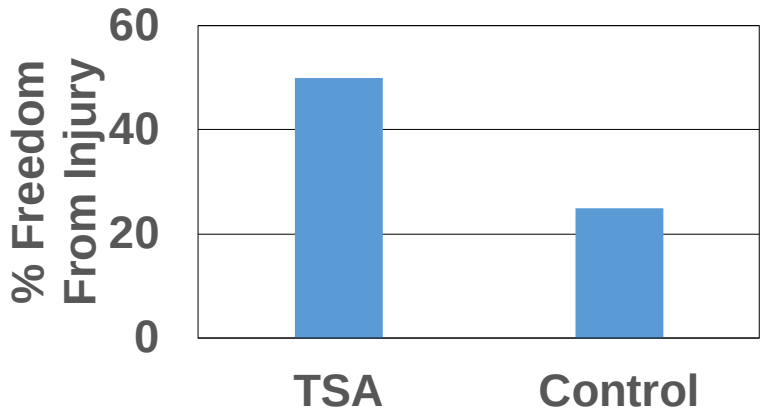
After obtaining regulatory approval, we have focused on the development of reproducible warm and cold ischemia models for limb (VCA) ischemia. (task 1&2; aim 1).

The subsequent work we have worked on development of a reproducible warm limb ischemia model – we have achieved this with a selected ischemia time of 60 mins and we have determined consistency of injury with readouts of pathology. We experienced a significant lead-in time to develop consistent histologic/pathologic readouts of limb IRI and attempted multiple parallel approaches to ischemia time and methodology. We have not found that nitro-blue tetrazolium (NBT) has yielded consistent results in scoring muscle fiber necrosis percentages and we initially had difficulty with adequate decalcification of the bony aspects of the limb sections to yield consistent high fidelity scoring results. We have recruited the core clinical pathology laboratory at CHOP to centralize the production of histology specimens and this has dramatically improved fidelity of tissue handling and has allowed us to proceed with data acquisition. We have used double blinded scoring of two pathologists to assess consistency of data. We will continue to explore NBT staining but are using the Baumeister method to score limb IRI (muscle necrosis graded categories, single vs multiple clustered fibers, granulocyte infiltration grade). In addition to this we have validated that serum myoglobin by ELISA correlates with degree of muscle injury and we have strored serum from early cases to run this as a secondary marker once histological testing is complete (Abcam kit). We have also identified that renal function may be used as a tertiary endpoint for severe muscle injury but renal injury is only noted with muscle necrosis rates that are in the higher categories of the Baumeister scale.

The CY 2017 tasks focused on the assessment of the impact of drug inhibition (trichostatin or TSA, which is a pan-HDAC inhibitor and MS-275, which is a class I HDAC inhibitor) as well as assessing the impact that HDAC 1 and 2 knockouts have on limb warm ischemia tolerance. An entire surgical experiment can be conducted in a single day (16 warm ischemia inductions with rubber band ligation is rapidly produced) but the tissue processing and scoring of samples takes 4-6 weeks so the iterations of working out optimal conditions has taken somewhat longer time than expected. We have completed assessment of pan-HDAC inhibitor TSA and this shows mitigation of warm IRI damage that approached significance. We have doubled the numbers of cases that will be assessed in subsequent experiments. We note that TSA treatment led to 50% rate (4/8) of freedom from muscle necrosis in the treatment group compared to 25% (2/8) in the control animals. We have performed the warm IRI experiments for the MS-275 animals and controls and these are awaiting final scoring. We have performed the IRI experiments for the HDAC-2 knockout mice compared to control this week and scoring will take 4-6 weeks to complete. HDAC-1 knockout experiments will be done within the next month and scoring will be completed subsequently. Serum myoglobin will then be assessed. This puts us on track to complete warm IRI experiments in early CY 2018. We conclude that pan-HDAC inhibition with drug has promise in limb warm IRI and further refinement of this conclusion awaits more data acquisition.

The cold ischemia (hindlimb orthotopic transplant) model has been surgically perfected and experiments will be initiated once the optimal beneficial strategy to mitigate warm IRI is identified so that cold ischemia experimentation can be aimed at the most highly leveraged approach.

In addition to the previously delineated studies, we plan to test tubastatin (an HDAC6 inhibitor) and a proprietary HDAC8 inhibitor as tubastatin has recently been shown in our laboratory to mitigate liver warm IRI and HDAC8 deletion and inhibition has been shown in our lab to improve renal IRI. These will first be tested in warm limb IRI models.



What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The TSA warm IRI data above will be presented at the 13th ISVCA meeting in Salzburg, Austria on October 26, 2017.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next year of the project, we expect to complete all proposed experiments with perhaps the exception of the additional testing of HDAC6 and 8 inhibitors which were not part of the original proposed work. We have already performed the surgical warm ischemia experiments testing MS-275 and HDAC2 knockout and will complete HDAC1 knockout experiments in the coming months. The final tissue processing and scoring will be largely complete in the next quarter. We will complete secondary endpoint analysis with serum myoglobin and renal function testing.
We will initiate cold ischemia testing via limb transplantation model on schedule. at the beginning of CY2018 focusing either on TSA or whichever strategy produces the greatest benefit on limb warm IRI as we have previously noted that strategies the benefit warm ischemia have translated to cold ischemia benefit in renal models in our laboratory.
We will test HDAC6 and HDAC8 inhibition pathways on warm ischemia and possibly cold ischemia in addition to those originally proposed based on data we have generated outside the scope of this proposal in liver and renal IRI.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

This line of inquiry sets to elucidate whether HDAC manipulation can impact how a donated limb may tolerate procurement, transportation, preparation, and transplantation while blood flow is interrupted. This work in limb transplantation mirrors efforts in my laboratory that investigate kidney and liver injury models and which have already spurred a clinical interventional trial in renal transplant patients in which estrogen administration will be tested for IRI mitigation. Our initial results indicate that pan-HDAC inhibition can improve the limb's tolerance of ischemia and this is a good early step in the process of eludcidating this mechanism and considering clinical translation.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

There have been no significant changes in approach although a significant effort has been enjoined to optimize the limb warm IRI system and improve the reliability of the readouts in terms of pathology and biochemical endpoints. After significant experimentation with method, we have put aside nitro-blue tetrazolium (NBT) staining as a primary endpoint for this time as we did not feel we had reliable and consistent staining with this agent and have elected to use the Baumeister method to score pathology along with adjunctive serum myoglobin and renal function testing. The recruitment of the core CHOP pathology laboratory has greatly enhanced the consistency and the quality of the limb histology sections and should allow us to re-explore NBT staining as an adjunctive measure.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We had somewhat greater lead-in time to perfect the preparation of histology sections as noted above but this issue has been resolved. We elected to defer large scale experimentation until the system was worked out as the surgical throughput in the warm ischemia model is very high but the tissue processing is slow and this would allow us to perform a large number of costly experiments quickly which may be fruitless if the system is not optimized. Now that we are attaining consistent results, we have completed TSA, MS-275, and HDAC2 knockout warm ischemia experiments and await scoring in the second two series. We expect to complete the warm ischemia experimentation with only a slight delay and will then select best-candidates for cold ischemia testing and we do not anticipate significant delays or problems in completing the work on schedule.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The recruitment of the core CHOP histology laboratory for tissue handling was cost neutral as we already had per-sample costs arranged for prior work that is in keeping with the budget. No other major costs changes are notable. We have not spent all budgeted funds for year 1 but we have not yet completed all of the budgeted work proposed in year 1 either and CHOP animal charges have not yet completely been paid for the animals on this protocol so we are at this time quite close to budget and expenditures are likely to match budget going forward.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report. CHOP IACUC Approval (IAC 16-000954) date for 3 year renewal of protocol: Jan 4, 2017.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Not at this time.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript*

• Levine MH, Concors S, Wang Z, Ge G, Murken D, Aufhauser Jr. D, Bhatti T, Levin LS, Hancock WW. "Histone Deacetylase Inhibition Mitigates Limb Ischemia Reperfusion Injury in Mice. 13th ISVCA Meeting, Salzburg, Austria. Oct 26-27, 2017. International.

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

None to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

The use of HDAC inhibitors in the setting of ischemia reperfusion injury in any clinical scenario is novel. We have published one paper in renal IRI and have ssubsequent manuscripts on renal and liver IRI in preparation. This application to limb ischemia, as it becomes further elucidated, will be publicized and shared through abstracts and publications to the scientific community.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*

- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

None to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Penn:
Matthew Levine – PI -- ORCID 0000-0003-4325-5827 – design, implementation of experiments and prep of regulatory submission – 1 month
Zhonglin Wang – technician – microsurgery and animal model optimization – 1 month
Guanghui Ge – technician – animal colony maintenance, tissue fixation and staining – 1 month
Scott Levin – consultative support and VCA surgical advisory capacity – 0 months
Seth Concors – surgical resident – animal model experimentation (warm ischemia) – 2 months
CHOP:
Wayne Hancock – Sub-PI – ORCID 56438952900 – design, interpretation, and standardization of experiments – 1 month

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Children's Hospital of Philadelphia (CHOP)
Philadelphia, PA, USA
Planned partner and both the PI and sub-PI have academic appointments there. The tissue processing has been centralized in the core pathology laboratory at CHOP and the histological interpretation is being performed by Dr. Hancock and Dr. Tricia Bhatti at CHOP.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Improving Ischemia Reperfusion Injury in Vascularized Composite Tissue Allotransplantation Via Histone Deacetylase Modulation

DoD Idea Discovery Award W81XWH-15-RTR-IDA
RT150071

PI: Matthew H Levine **Org:** University of Pennsylvania **Award Amount:** $450,000.00

Study/Product Aim(s)

1) Does Class I HDAC inhibition or deletion impact limb warm ischemia reperfusion injury (IRI) tolerance?

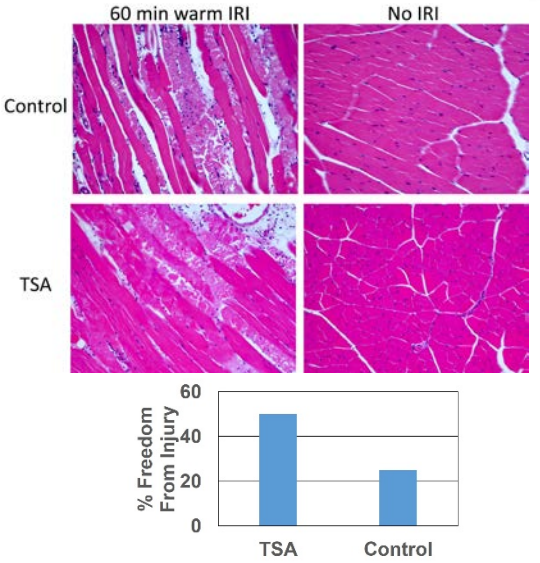
2) Do HDAC inhibition or deletion benefits in limb warm ischemia translate to cold ischemia tolerance in murine models of VCA?

3) Are the possible benefits of HDAC manipulation in limb transplantation gained by inhibiting HDACs in the donor, the recipient, or a combination of the two?

Approach

We proposed proof-of-concept studies in murine warm limb ischemia and cold ischemia transplantation (VCA) models in mice treated with histone deacetylase (HDAC) inhibitors or in mice where HDACs have been knocked-out in donor or recipient to test the effect of HDAC inhibition or deletion on the tolerance of limb warm and cold ischemia.

We have initiated a series of warm IRI experiments comparing TSA with DMSO control for limb injury (treatment 16h and 30m before IRIl; limb histology assessed at 24h after reperfusion). We identify that non-ischemic contralateral limbs develop inflammation at 24h but no damage that is similar in treated and untreated groups (R panels) and that TSA treatment has led to diminished myocyte swelling, hyalinization, and nuclear loss compared to control (bottom row compared to top two rows). TSA treated mice had no identified muscle injury in 50% of samples (4/8) compared to 25% (2/8) in untreated mice.



Timeline and Cost

Activities / CY	16	17	18	
Regulatory approval & start warm IRI modeling				
Test effects of HDACi/HDAC ko on Warm IRI				
Test effects of HDACi/ HDAC ko on cold IRI (Transplant) model				
Assess Donor and Recipient HDAC effect and publish				
Estimated Budget ($K)	**$000**	**$225K**	**$225K**	

Updated: Oct 25, 2017

Goals/Milestones

CY16 Goal – Obtain regulatory approval and establish warm IRI model
☑ IACUC and ACURO approval
CY17 Goals – Test effects of HDACi and HDACko on warm & cold limb IRI
☑ Develop warm ischemia model
☑ Develop pathology and laboratory based assays for limb injury
☐Test IRI in HDACi & HDACko mice with warm IRI (HDAC1ko remains)
☑ Develop cold (transplant) limb IRI model (complete)
CY18 Goal –Test donor specific effects of HDACi & HDAC ko on limb IRI
☐Cold (transplant) IRI experiments with HDACi & HDAC ko (started)
☐Assess donor/recipient contributions to HDACko effects on IRI
☐Publish the results of our studies

Comments/Challenges/Issues/Concerns

- No concerns.
- Histology optimization has taken some time but has been achieved through use of the CHOP core histology laboratory

Budget Expenditure to Date

Projected Expenditure: $225,000 direct and indirect
Actual Expenditure: $177,830 direct and indirect

Histone Deacetylase Inhibition Mitigates Limb Ischemia Reperfusion Injury in Mice

Matthew Levine, Seth Concors, Zhonglin Wang, Guanghui Ge, Douglas Murken, David Aufhauser Jr, Tricia Bhatti, L. Scott Levin, and Wayne Hancock

Departments of Surgery/Orthopedics/Pathology, University of Pennsylvania / Children's Hospital of Philadelphia, Philadelphia, PA, USA

Introduction

- Vascularized composite allotransplantation (VCA) of the limb is accompanied by concomitant combined cold and warm ischemia that limits travel and ischemia time and may limit organ availability to a particular donor that must be matched generally by blood type, HLA compatibility, age, size, skin color, and gender.
- No current therapy exists to mitigate warm or cold ischemia injury and subsequent ischemia reperfusion injury (IRI) in solid organ transplantation or VCA.
- Histone deacetylases (HDACs) regulate diverse cellular processes. HDACs are divided by homologous structure/function: Class I (HDAC-1, -2, -3, -8), Class IIa (HDAC-4, -5, -7, -9), Class IIb (HDAC-6, -10), Class IV (HDAC 11).
- Pharmacologic HDAC inhibitors include: Pan-HDAC inhibitor Trichostatin A (TSA), Class I inhibitor MS275, and HDAC-6 specific inhibitor Tubastatin A (TubA)
- We have previously demonstrated that pan-HDAC inhibition with trichostatin (TSA) and Class I HDAC inhibition with MS275 mitigates IRI in models of renal and hepatic IRI[1].
- Pathologic correlates of ischemia reperfusion injury include muscle necrosis, inflammatory cell in-migration, and interstitial hemorrhage.

Methods

- Female wild type C57BL/6 (WT) mice treated with TSA (1mg/kg), or control (DMSO) at 16 hours and 30 minutes pre-IRI were used in the IRI experiments.
- Mice underwent 60 minutes of limb ischemia under strict temperature control (37.0 +/-0.5ºC), with the contralateral limb serving as internal control (Figure 1).
- At 24 hours post-IRI portions of ischemic limb, and control was harvested and paraffin embedded for histopathology.
- Histopathology was scored in a blinded fashion on an accepted scale for amount of muscle necrosis[2, 3]
- Muscle necrosis amount (0: none, 1: 1-33%, 2: 34-66%, 3: 67-100%), Muscle necrosis pattern (1: single area, 2: grouped), granulocyte demarcation (0: none, 1: mild, 2: marked, 3: severe infiltration)

Figure 1. A dental band is placed just proximal to the knee joint under general anesthesia, which creates total vascular occlusion of the hind foreleg for 60 minutes, followed by the lysis of the band and subsequent immediate reperfusion.

Histopathology

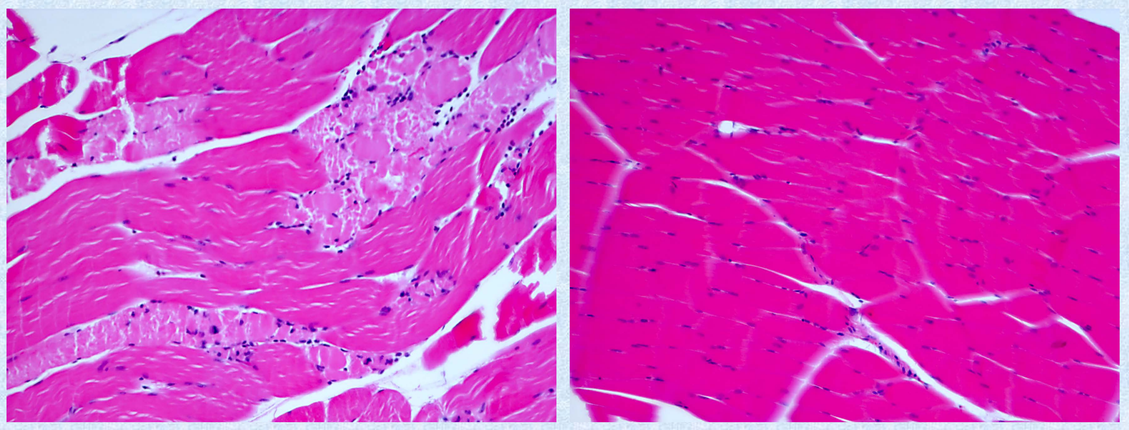


Figure 2a. H&E sections of control– Ischemic limb (L) demonstrating **mild** ischemic injury, versus internal control non-ischemic limb (R)

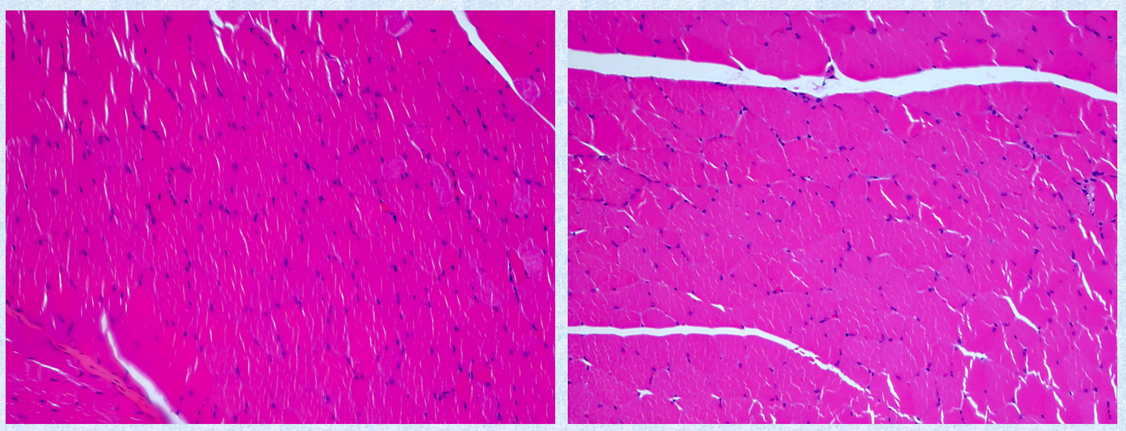


Figure 2c. H&E sections of TSA– Ischemic limb (L) demonstrating **negligible** ischemic injury, versus internal control non-ischemic limb (R)

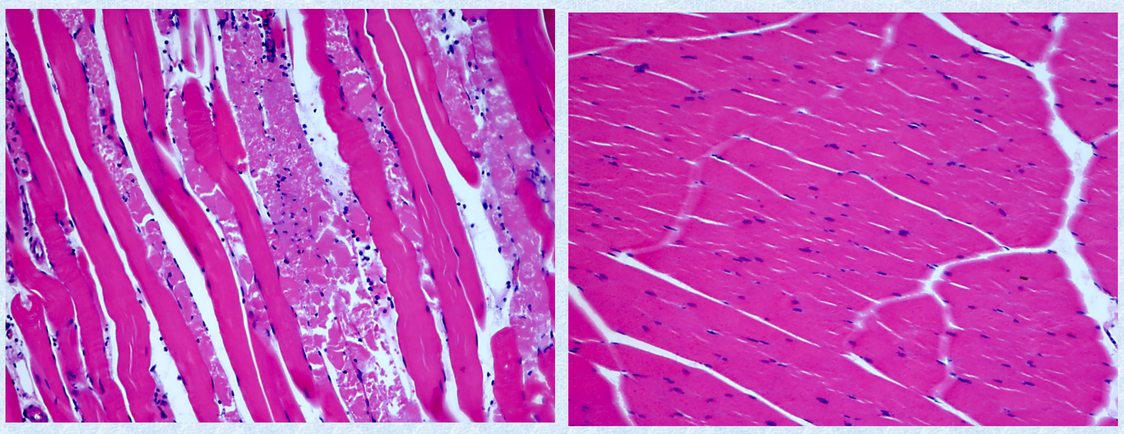


Figure 2b. H&E sections of control– Ischemic limb (L) demonstrating **moderate** ischemic injury, versus internal control non-ischemic limb (R)

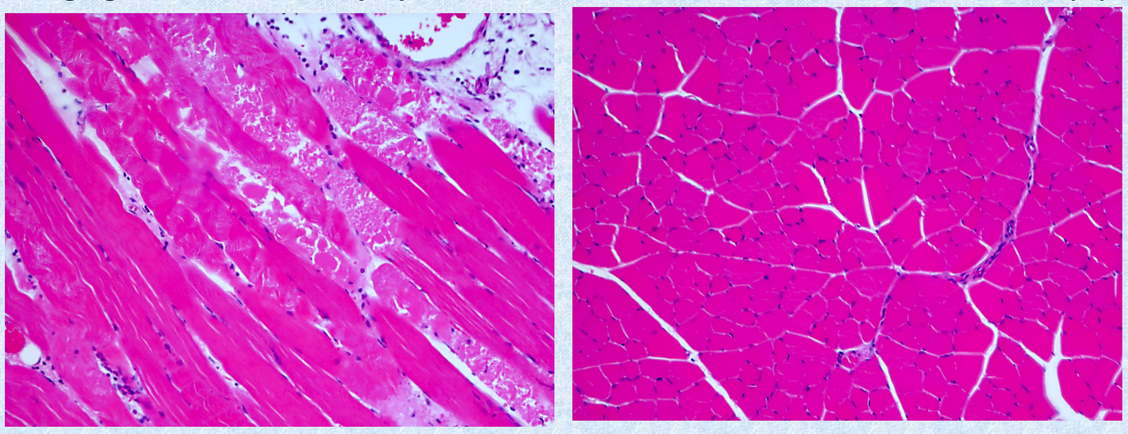


Figure 2d. H&E sections of TSA– Ischemic limb (L) demonstrating **moderate** ischemic injury, versus internal control non-ischemic limb (R)

Conclusions

- A reproducible level of ischemia is possible with the application and lysis of an elastic band just below the knee joint.
- IRI is mitigated by the administration of the HDAC inhibitor, evidenced by histopathologic difference (Figure 2) and freedom from injury - 50% TSA versus 25% Control (Figure 3)
- Additional studies will test class- and isoform-specific HDAC inhibitors, as well as determine HDACi impact on cold ischemia in the setting of limb transplantation in mice

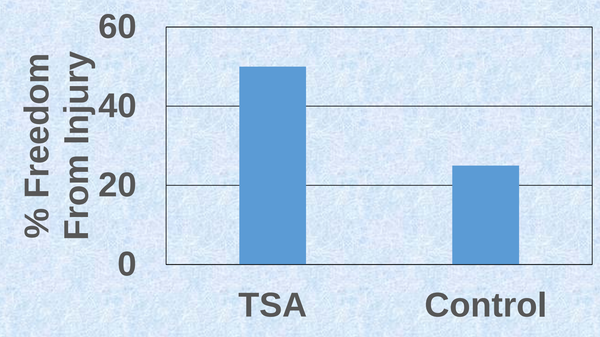


Figure 3: 50% of subjects in TSA group (n=4) demonstrated freedom from histologic injury, relative to 25% in the control group (n=2)

1. Levine et al. *Am J Transplant*. 2015;15(4):965-73
2. Hautz et al. *Transplantation.* 2014; 98(7): 713-20
3. Baumeister et al. *J Reconstructive Microsurg*. 2004; 20(3) 253-9